a significant reduction of resting calcium concentration in both muscle types due to decreased sarcolemmal permeability at rest. Our results indicate that PKC-theta contributes to the regulation of ClC-1 channel differently in the muscle types and that this isoform can also modulate calcium homeostasis likely by interacting with sarcolemma channels. (ASI-OSMA)

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Interleukin-1β Decreases Aquaporin-3 Expression via Trans-Repression by CCAAT Enhancer Binding Protein

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Aquaporin-3 (AQP3), which is expressed in the basal layer of keratinocytes and airway epithelial cells, regulates not only water and glycerol permeability at plasma membrane, but also cell migration. We have previously shown that the expression of AQP3 is decreased by inflammatory cytokines, such as TNF-α and IL-1β. However, underlining mechanism for this decrease in AQP3 remains to be clear. In the present study, therefore, we examined the effect of IL-1β on AQP3 mRNA expression and promoter activity in A549 lung epithelial cells. IL-1β decreased both AQP3 protein and mRNA expression, in a concentration- (0.5-50 ng/ml) dependent manner. IL-1β also decreased promoter (−990/+88) activity, suggesting that IL-1β repressed AQP3 gene transcription. The IL-1β-induced decrease in AQP3 was relieved with PD98059, a MEK/ERK inhibitor. We then prepared a series of 5'-deletion constructs of AQP3 promoter. Among these, IL-1β did not decrease the activity of mutant promoters without −374/-272 region. The promoter, which has point mutation at CCAAT enhancer binding protein (C/EBP) binding element located at −276/-262, was also insensitive to IL-1β. Taken together, it was suggested that IL-1β decreases AQP3 expression via MEK/ERK- and C/EBP-dependent signaling.

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Inhibition of a Dimer Interface Mutant of CLC-0 by Intracellular Cadmium Ion

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The CLC chloride-transporting proteins play a variety of physiological roles: regulate the cellular excitability in skeletal muscle cells, facilitate the electrolytes transport in epithelial cells and control the pH in intracellular compartments. These membrane proteins are homodimers with each molecule containing two identical subunits. A double-point mutation (I201W/I422W) at the dimer interface in the bacterial CLC molecule (CLC-ec1) has recently been shown to destablize the dimer interaction so that the functional unit of the I201W/I422W mutant of CLC-ec1 is a monomer. We made the corresponding double mutant at the dimer interface of CLC-0, and the fluorescence resonance energy transfer (FRET) experiments indicated that the mutant channel still consists of two subunits. This dimer-interface mutant of CLC-0, however, shows very different functional properties in comparison to the wild-type CLC-0: the mutant channel is activated only at hyperpolarized voltages. Furthermore, the mutant channel significantly enhances the inhibition of the channel by the intracellularly-applied cadmium ion (Cd^{2+}). The Cd^{2+} inhibition of this dimer-interface mutant appears to be state-dependent with the closed-state channel being more sensitive to the Cd^{2+} inhibition than the open-state channel. We are currently mutating the endogenous cysteine and histidine residues of CLC-0 to identify the potential Cd^{2+}-binding site.

Biophysics of Ion Permeation

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Recharging the Phylogenetic Analysis of Voltage Sensor Domains

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Sequence and phylogenetic analysis of voltage-gated ion channels has famously resulted in the discovery of novel ion channels in previously unexplored phyla, and has provided insight into the molecular underpinnings of ion channel function. Because of their crucial role in human health, most current evolutionary analysis has quite reasonably centered on 'traditional' pore-forming channels (e.g., Yu, et al, 2005, Pharmacol. Rev., 57:387-395, among many others). Earlier phylogenetic analyses of voltage sensor domain (VSD) modules (e.g., Komanovics et al, 2002, FASEB J., 16:1623-1629, among others) were undertaken before the discovery and characterization of the genes for voltage sensitive phosphatases (VSP) and voltage gated proton channels (HV1), which contain VSDs but do not contain traditional ion pores. We recently reported two new discoveries: in one line of inquiry we found and characterized a dinoflagellate HV1, supporting the prediction of its existence in bioluminescent dinoflagellates by Fogel and Hastings in 1972 (PNAS, 3:690-693); and in the other we uncovered the universal selectivity filter of HV1's. Both of these studies were informed by large-scale sequence and phylogenetic analyses that focused on homologs of VSDs themselves, separate from their N- or C-terminal appendages. Here we present a full sequence and phylogenetic analysis that updates earlier work and reveals that VSDs may have taken a different evolutionary path from associated ion channel pore domains, which has mechanistic and physiological implications.

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Role of Central Cavity in Ion Permeation through the Kv1.2 Channel

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In the ion permeation pathway of the K^+ channel, the narrow selectivity filter is a critical region where ions and water molecules permeate with a single-file fashion. Theoretical studies have been concentrated on the permeation process in the selectivity filter, whereas the role of the central cavity, a common architecture that all the known K^+ channels have, is not systematically examined yet. Here we investigated ion permeation through the Kv1.2 channel by the molecular dynamics simulation and analyzed the role of the central cavity. It is found that the central cavity attracts the K^+ ions when the ion concentration in the bulk is below the physiological range. Accordingly, the concentration in the central cavity is higher than that in the bulk, increasing the entry chance of ions into the selectivity filter. Thus, the central cavity plays a role to make ion permeation rapid. On the other hand, the ion concentration in the central cavity is found to be saturated at high concentration in the bulk, and the conductance is saturated. These results indicate that the concentration of ions in the central cavity generates the Michaelis-Menten-type behavior of the conductance-concentration curve in the Kv1.2 channel. In contrast, it is known that the saturation of the conductance is not observed in the KcsA channel. We found that the ion concentration in the central cavity increases progressively as the bulk concentration increased up to 2 M, which is in agreement with the apparent non-saturating concentration-conductance curve in the previous studies.

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Interpreting the Barium Blockades of Potassium Channels with the Multi-Ion Permeation Free Energy Surface

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Barium blockade experiments on the BK channel provided an early quantitative determination of the ion binding selectivity of a potassium channel (Neyton, Miller, *J. Gen. Physiol.* 1988, 569). Recently, these experiments were repeated for KcsA by Piasta and Miller, providing the first site selectivity electrophysiological measurements for a channel of known atomic structure (Piasta, Theobald, Miller, *J. Gen. Physiol.* 2011). Site selectivity can be determined from these blockade experiments because the binding of an external alkali cation in the so-called lock-in site impedes the translocation of Ba^{2+} toward the external side, thus increasing the length of blockade. As K^+ and Na^+ impede the rate of Ba^{2+} translocation to vastly different degrees, their relative binding affinities to the lock-in site can be determined quantitatively. Here, we have used molecular dynamics simulations of KcsA to model the permeation process of Ba^{2+} by computing the ion permeation potential of mean force (PMF) with umbrella sampling enhanced by Hamiltonian exchange. Although a Ba^{2+}-bound crystallographic structure (Lockless, Zhou, MacKinnon, *PLoS Biology*, 2007, 5, e121) showed Ba^{2+} binding in the S4 and S2 sites, we find that Ba^{2+} can bind in all five internal sites, with site binding affinities ranked in the order: S2 > S1 ≈ S3 > S0 ≈ S4. Permeation of Ba^{2+} in the absence of external K^+ proceeds through the familiar knock-on mechanism, where the Ba^{2+} moves in concert with an internal K^+ ion. The barriers for the transition between binding sites are high (>15 kcal/mol), consistent with the observation of long-lived channel blockades and slow permeation of Ba^{2+} relative to K^+. The K^+ lock-in effect is examined by a multi-ion PMF of Ba^{2+} permeation in the presence of an external K^+ ion that impedes the permeation of Ba^{2+}.